

### **REMARKS**

Claims 1-35 are pending. Claims 13-35 are withdrawn for being drawn to a non-elected invention. Claims 2 and 4 are cancelled. Claim 1 is amended. New claims 36-40 are added. No new matter is added.

Claim 1 has been amended to replace the term “enzyme” with the term agent. New claims 36-40 relating to specific agents have been added.

Support for amended claim 1 and new claims 36-40 is found throughout the specification and in particular at page 5, lines 22-25: “[t]he present invention provides a microfluidic device capable of reacting an enzyme or other agent with a substantially purified polypeptide; page 25, lines 5-8: “[i]n one embodiment of the present invention, the first sample is treated with a particular enzyme or derivatizing agent and the second sample is sent directly to a peptide analysis module 17 to be analyzed. In one embodiment of the present invention, the particular enzyme is a phosphatase”; page 25, lines 17-19: “[i]n one embodiment of the present invention, the first sample of the substantially purified polypeptide is treated by a cross-linking enzyme and the second sample is not treated by a cross-linking enzyme”; page 25, lines 23-25 “[t]hose of skill in the art will recognize that many enzymes and/or derivatizing agents are within the spirit and scope of the present invention”; and at page 26, lines 8-19:

“[i]n a preferred aspect, proteases are contained within one or more of the reaction channels 8 of the microfluidic device 5. Suitable proteases include, but are not limited to: peptidases, such as aminopeptidases, carboxypeptidases, and endopeptidases (e.g., trypsin, chymotrypsin, thermolysin, endoproteinase Lys C, endoproteinase GluC, endoproteinase ArgC, endoproteinase AspN). Aminopeptidases and carboxypeptidases are useful in characterizing post-translational modifications and processing events. Combinations of proteases also can be used. Where the system comprises a plurality of reaction channels 8, at least one channel can be free of proteases and/or resistant to protease digestion (e.g., can comprise one or more protease inhibitors as described above). Further, different channels can comprise different types or amounts of protease or other enzymes or derivatizing chemicals to perform a plurality of reactions of substantially identical samples (e.g., obtained from a single sample

plug) in parallel. Agents for sequence-specific cleavage also can be provided such as, and the like.”

Claims Rejected Under 35 U.S.C. §103(a):

Claims 1, 3 and 5-12 remain rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over U.S. Patent No. 6,007,690 to Nelson et al. (“Nelson”) in view of Wang et al. (Rapid Communications in Mass Spectrometry) (“Wang”).

Applicant respectfully traverses the rejection.

For the reasons described below, Applicants respectfully submit that the Examiner has failed to establish a *prima facie* case of obviousness under the requirements of 35 U.S.C. § 103(a). To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings (*In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). Second, there must be a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on Applicants’ disclosure. Finally, the prior art reference (or references when combined) must teach or suggest ***all the claim limitations***. *In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974).

The Examiner states at pages 3-4 that:

“With respect to the specifics of the membrane employed of claims 1 and 3, the reference of Nelson et al. discloses a number of possible supports that can be employed with respect to the enrichment channel (See column 6, lines 1-56). Specifically, the reference of Nelson et al. discloses the use of “ion-exchange membranes” (See column 6, lines 37-45). An ion-exchange membrane is charged and can have pores that are larger than the charged analyte that it binds with since it is merely functioning as a support matrix for binding rather than a physical particle filter. As a result, in the absence of a showing of criticality and/or unexpected results, it would have been obvious to one of ordinary skill in the art at the time the invention was made to determine the optimum material for enclosing the enrichments channel based merely on the specifics of the analyte to be reacted and/or detected in the system.”

The Examiner also states at page 5, that “the reference of Wang et al. discloses that it is conventional in the art to provide the protein digested sample of a microfluidic device to a MS for further separation and analysis...As a result, it would have been obvious to one of ordinary skill in the art at the time [of] the invention was made to further analyze the reaction products of the device of Nelson et al. using a MS as suggested by the reference of Wang et al.

Amended claim 1 claims “a microfluidic device, comprising: an inlet channel; at least one reaction channel engaged to the inlet channel wherein an agent is located within the reaction channel; at least one membrane having a plurality of pores in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte; and an electric field generating device capable of applying an electric field of a selected polarity to said membrane during the concentration of the analyte, the electric field generating device being capable of generating an electric field of the opposite polarity to said membrane to allow removal of the concentrated analyte from said membrane.**” (Emphasis added)

Support for the inclusion of “an electric field generating device” in amended claim 1 is found throughout the specification and in particular at page 6, lines 11-16:

“[i]n a preferred embodiment of the present invention, the system is driven by electroosmotic flow. In a preferred embodiment, a negative electrode is positioned adjacent to the positively charged membrane and a positive electrode is positioned adjacent to the negatively charged membrane. In a preferred embodiment, a charge trapping mechanism is developed at each membrane allowing analytes to be concentrated at a membrane wherein the diameter of the analyte is smaller than the diameter of the pore of the membrane”;

page 10, lines 3-8:

“[i]n one aspect of the present invention, a membrane was integrated into a microfluidic device for the purpose of concentrating analytes. In a preferred embodiment of the present invention, a nanocapillary array (or nanochannel array) is integrated into a microfluidic device for the purpose of

concentrating analytes. Through the application of an electric field across the channel, charged analytes were concentrated in front of the membrane, and a concentrated analyte band was ejected from the channel by reversing the polarity of the electric field”;

and page 10, lines 17-25:

“For electrically driven concentration, analyte retention in front of the membrane appears to occur primarily by a charge trapping mechanism. In the presence of such a charge trapping mechanism, relatively large pores can be used to concentrate the small molecules, making the system more robust. The only known limitation to the buffer system is that the conductivity must be low enough to prevent current breakdown, and there is no need for multiple buffers or solvents which most concentrating for microfluidics and capillary electrophoresis require. Not only can this device be used for analyte concentration, but it can be used as a concentrating micro-reactor as many species can be co-localized in front of the membrane. With this simple and robust design, concentration factors of 300-fold have been achieved.”

Nelson discloses a microfluidic device comprising at least an enrichment channel and a main electrophoretic flow path positioned such that waste fluid flows away from the main electrophoretic flowpath through a discharge outlet. The enrichment channel serves to enrich a particular analyte comprising fraction of a liquid sample. In particular, the enrichment channel serves to selectively retain and separate the target analyte comprising fraction from the remaining components or the waste portion of the initial sample volume. The Nelson reference teaches a variety of different enrichment media that may be present in the enrichment channel, for example, sorptive phase materials (see column 5, line 12 through column 6, line 53). The Nelson reference teaches the use of membranes comprising sorptive materials as an enrichment material useful according to the invention (column 6, lines 30 through 53). The Nelson reference also teaches that an elution liquid is required to release the enriched sample fraction from the enrichment material (see column 7, lines 2-5).

The Wang et al. reference describes a microfluidic device comprising trypsin-loaded beads in a microfluidic chip for digestion of peptides and proteins.

**Even if the references are combined, they do not provide the invention as claimed.**

Applicant submits that even if Nelson is combined with Wang the two disclosures do not provide the invention as claimed in claims 1, 3 and 5-12. That is, the recited combination lacks essential elements of the claimed invention.

The instant claims require “an electric field generating device capable of applying an electric field of a selected polarity to said membrane during the concentration of the analyte, the electric field generating device being capable of **generating an electric field of the opposite polarity to said membrane to allow removal of the concentrated analyte from said membrane.**” (Emphasis added)

None of Nelson, Wang or their combination teach or suggest “an electric field generating device capable of applying an electric field of a selected polarity to said membrane during the concentration of the analyte, the electric field generating device being capable of generating an electric field of the opposite polarity to said membrane to allow removal of the concentrated analyte from said membrane”, as required by the instant claims.

Nelson teaches analyte retention on an enrichment medium that is a membrane comprising a sorptive material, for example an ion-exchange membrane (see column 6, lines 30-53), wherein the analyte is removed from the membrane by the addition of an elution buffer (see column 7, lines 2-5). That is, Nelson does not teach or suggest using an electric field device to generate an electric field of the opposite polarity to a membrane to allow removal of the concentrated analyte from the membrane, as required by the instant claims. The Wang reference does not cure this deficiency.

Nelson describes using an electric field to move fluid through the enrichment channel and subsequently using an elution liquid, which flows through the enrichment medium, to release the enriched sample fraction from the material (See column 6, line 66-column 7, line 5). However, Nelson does not teach or suggest “an electric field generating device capable of applying an electric field of a selected polarity to said membrane during the concentration of the analyte, the electric field generating device being capable of **generating an electric field of the opposite polarity to said membrane to allow removal of the concentrated analyte from said**

**membrane**", as required by claims 1, 3, 5-12 and new claims 36-40. The Wang reference does not cure this deficiency.

The novelty of the instant claims is based, in part, on the discovery that under certain conditions analytes that are smaller than the pores of a membrane can be concentrated by the membrane. The instant specification states at page 6, lines 14-18 that "In a preferred embodiment, a charge trapping mechanism is developed at each membrane allowing analytes to be concentrated at a **membrane wherein the diameter of the analyte is smaller than the diameter of the pore of the membrane**. Such a result cannot be achieved with a hydrodynamic flow system. In one embodiment, a nanocapillary array (or a nanochannel array) is utilized to concentrate the respective analytes." (Emphasis added).

The specification also states at page 10, lines 11-14 that "In one aspect of the present invention, in the presence of an electric field a charge trapping effect was observed; small molecules can be concentrated in front of **membranes with pore sizes which are orders of magnitude above the molecular weight cut-offs for hydrodynamically driven systems.**"

The specification also states at page 10, lines 17-25 that "For electrically driven concentration, analyte retention in front of the membrane appears to occur primarily by a charge trapping mechanism. In the presence of such a charge trapping mechanism, **relatively large pores can be used to concentrate the small molecules**, making the system more robust. The only known limitation to the buffer system is that the conductivity must be low enough to prevent current breakdown, and there is no need for multiple buffers or solvents which most concentrating for microfluidics and capillary electrophoresis require. Not only can this device be used for analyte concentration, but it can be used as a concentrating micro-reactor as many species can be co-localized in front of the membrane. With this simple and robust design, concentration factors of 300-fold have been achieved."

None of Nelson, Wang or the combination thereof teach or suggest a microfluidic device comprising "at least one membrane having a plurality of pores in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the

reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte**” as required by the instant claims.

In fact, Nelson et al. teaches away from a “membrane having a plurality of pores in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte.**”

At column 13, lines 40-45 of Nelson it is stated that “[i]n device **90**, instead of the stacking gel configuration, one could provide for a molecular size membrane at the region of interface **93**, which can provide for selective passage of sample components below a threshold mass and retention at the membrane surface of components in excesses of the threshold mass.” Thus, Nelson clearly does not teach a membrane having a plurality of pores in communication with the reaction channel capable of concentrating a charged analyte produced by a reaction in the reaction channel, **wherein the pores of the membrane have a pore diameter greater than a diameter of the charged analyte**” as claimed in claims 1, 3 and 5-12 of the instant application. It is well settled law that citing references which expressly lead away from what the Patent Office contends is obvious therefrom is improper. In re Grasselli, 218 U.S.P.Q. 769 (Fed. Cir. 1983). It is also established in the law that a claimed invention cannot be found obvious in view of a reference that leads one skilled in the art away from the claimed invention. In re Dow Chemical Co. v American Cyanamid Co. (CAFC) 2 U.S.P.Q. 2d 1350.

It is submitted that one of skill in the art would not be able to combine the system of Nelson with the system of Wang so as to successfully arrive at the invention as described in the instant claims because neither of Nelson or Wang or their combination teaches or suggests the essential elements of the claims.

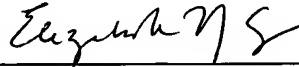
In view of the above, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 1, 3 and 5-12.

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. If the Examiner believes that a telephone conversation with

Applicant's attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record.

Respectfully submitted,

Date: September 6, 2006



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